

## A FISH DISEASE FROM GEORGE'S RIVER.

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In the middle of August there was received from the Fisheries Department the carcase of a bream which had been found floating, in a dying condition, on the surface of George's River, a few miles south-west of Sydney. The epidermis showed several slightly hæmorrhagic patches. The lateral blood vessels and adjacent portions of the muscles were congested; the liver was mottled and pulpy; the peritoneal cavity contained cheesy masses. The stomach was much congested and contained a reddish viscid fluid. The intestine was slightly congested near the anus, and contained a dark bile-coloured fluid. The other organs were apparently normal, and the muscles and alimentary canal were found to be free from parasites.

In the bacteriological investigation, plate cultures were made with media which had been inoculated with the several parts of the carcase. The muscles were proved to be sterile, while the heart blood, the spleen, and the liver contained three organisms. The first of these was a gelatine-liquefying fluorescent bacterium (*Bact. fluorescens*), which was not investigated further. The second was a rather large bacterium, which slowly liquefied gelatine and exhibited bipolar staining. A pure culture of this organism was distributed in normal saline, and a few drops of the suspension was injected into the muscles of two carp. Beyond showing a scar at the point of inoculation, these were unaffected and were apparently healthy after a month's observation. The organism was not investigated further.

The third organism was a smaller bacterium which rapidly peptonised gelatine and stained bipolarly. It occurred in

practically pure culture in the intestine, and with the other two bacteria in the organs. When suspended in normal saline and inoculated into the muscles of a carp, it strongly affected the experimental fish. In 24 hours its movements were slow; in 40 hours (the morning of the second day) it was found floating upon its side near the surface of the water of the aquarium, respiring rapidly and swallowing and ejecting air. It was apparently in a dying condition. Four hours later it had sunk to the bottom of the water and was respiring very slowly, still lying upon its side. On the morning of the third day it was found dead, floating upon the surface of the water. Death had probably taken place in 52 hours after inoculation.

No lesions were observed on the epidermis with the exception of a hæmorrhagic streak between the rays of the caudal fin. The muscles were very hæmorrhagic on the side upon which the carp had been inoculated, and which had been downwards for practically 24 hours. The muscle at the site of inoculation (midway between the anal fin and the posterior end of the dorsal fin) was a shade less hæmorrhagic than the surrounding muscle. The organs, with the exception of the kidneys, were apparently normal; these were pulpy and swarmed with the inoculated bacteria. A plate culture from this organ showed the bacteria in pure culture. Cultures which were apparently pure were obtained by stroke cultivation upon agar from the muscles on both sides, from the spleen, the liver, and the heart blood.

Since the organism, when inoculated into the experimental fish, caused it to float upon the surface of the water (a somewhat unusual symptom), and brought about death with a similar hæmorrhagic appearance of the muscles as occurred with the original fish, there is no reason to doubt that it also produced the death of the original bream. There are some differences in the lesions, but these may be accounted for by a difference in the species of fish or in the method of inoculation. To test the effect of feeding, a carp was given infected vermicelli, but no symptoms followed, and it is therefore probable that the original bream was

not infected by way of the alimentary canal, but by an accidental wound or a bite from another fish.

The organism was cultivated upon the usual media with the view of determining its name or its allies, and the actual appearances, etc., are here recorded for future guidance.

#### DESCRIPTION OF THE BACTERIUM.

*Shape, etc.*—It is a short rod with rounded ends. The size is variable, ranging from 0.45 to 0.7  $\mu$  in breadth, and from 1.2 to 1.5  $\mu$  in length. Longer forms may occur. In the animal tissues it has a distinct capsule. The organism stains feebly with methylene-blue, better with thionin-blue, and strongly with carbol-violet or carbol-fuchsin. With the latter stains many of the forms appear more or less bent and vibron-like. When stained with thionin-blue, with dilute fuchsin, or with carbol-fuchsin, followed by washing in alcohol, the younger organisms exhibit bipolar staining; the older and longer organisms stain in three places, at the poles and centrally. This latter appearance is probably caused by two organisms whose ends are close together while enclosed within a thin capsule. The cells are actively motile, the motion being produced by a single terminal flagellum, whose length varies from  $1\frac{1}{2}$  to 3 times the length of the cell. The organism does not appear to form spores.

*Gelatine plate.*—In 24 hours, at 22° C., the organism forms in the medium circular liquefied areas about 1.5 mm. in diameter. The colony shows a white central point and a white marginal ring. When magnified sixty-fold, there is seen an irregular brownish-black granular centre, then a clear portion containing large floating granular clumps, and finally similar large granules clustered around the margins. The appearance is like a colony of *Vibrio cholerae*. The edge of the colony is smooth. In 48 hours the colony widens to 5 mm., and the contents appear uniformly turbid.

*Gelatine stab.*—The medium is liquefied along the line of inoculation in 24 hours in a tubular manner, and an air-bubble

appears at the top. The liquefied medium is turbid, and there is a white deposit but no film. In 48 hours the medium is almost completely liquefied, the fluid is turbid, and there is a slight film. After a time (7 days) a turbidity extends from the surface to about 0.5 cm. downwards, the lower portion of the liquefied medium is clear, and there is a white granular deposit; no film is apparent.

*Glucose-gelatine or lactose-gelatine.*—No gas is produced in either of these media.

*Agar plate.*—The surface colonies are circular, translucent white, raised, and moist glistening. When magnified they are seen to be circular, finely granular, with isolated coarse granules in the centre; the margin is homogeneous and the edge smooth. The deep colonies when magnified are seen to be yellowish brown, coarsely granular, and irregularly shaped, an arrow-head pattern being most usually seen.

*Agar slope.*—At 22° the growth is translucent white and raised, with a slightly lobed or straight margin and smooth edge. It is spread out and irregular at the base when in contact with the condensed water. The latter is turbid and contains a sediment. At 37° the organism refuses to grow unless a considerable quantity of material is used for sowing. The growth is, however, never so luxuriant as at 22°.

*Bouillon.*—The medium becomes turbid, especially near the surface, upon which a slight film forms; there is a precipitate. The turbidity persists, and at the end of seven days a slight indol reaction can be obtained.

*Anaerobic culture.*—The organism grows in bouillon when placed in Buchner's tubes, but not so freely as in the presence of air.

*Nitrate-bouillon.*—The nitrate is strongly reduced in 4 days to nitrite.

*Litmus-milk.*—The casein partly dissolves and partly precipitates, while the litmus is first reddened and finally (7 days) bleached.

*Potato.*—There is a flat growth barely perceptible, being just sufficient to obscure the glistening surface of the medium.

The white colour of the colonies, the liquefaction of the gelatine, and the motility of the organisms, show that the bacterium has its closest allies in a group which consists chiefly of harmless water bacteria. The tendency to produce slightly curved or vibron forms is characteristic of the phosphorescent bacteria which form a subdivision of this group. The organism, however, does not produce phosphorescence when grown in sea water, in sea water with 1% peptone, in sea water gelatine or upon sterile fish muscle. Excluding the phosphorescence, the other characters show an affinity with the subdivision and, as far as the rapid liquefaction of the gelatine is concerned, with one of the members *Bacillus luminosus*, Beijerinck, which is identical with an organism described by Katz as *Bacillus argenteo-phosphorescens liquefaciens* and renamed *Vibrio luminosus* by Lehmann and Neumann. As the organism does not appear to have been previously described, I propose the name, following the nomenclature of Lehmann and Neumann, *Vibrio bresimæ* (low Latin—Bresmia, the bream).